Amyloidosis:

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Published in:
RHEUMATIC DISEASE CLINICS OF NORTH AMERICA

DOI:
10.1016/j.rdc.2013.02.012

Publication date:
2013

Amyloidosis: A Clinical Overview

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KEYWORDS

• Systemic amyloidosis • Amyloid fibril • Protein misfolding • Precursor protein • Typing • Diagnosis • Treatment • Disease monitoring

KEY POINTS

- Amyloidosis is the name for some diseases caused by protein misfolding; 30 different soluble precursor proteins can aggregate and be deposited as insoluble amyloid fibrils.
- Amyloid deposition is localized or systemic; the 4 main types of systemic amyloidosis are AL (light chain), AA (inflammation), ATTR (hereditary and old age), and $A\beta_2M$ (dialysis).
- Clinical management comprises proof of amyloid, systemic evidence, reliable typing, precursor assessment, severity of organ disease, choice of treatment, and planned follow-up.
- The precursor-product concept is the current basis of treatment, thereby aiming to decrease the levels of precursor proteins in serum to normal or undetectable values. Future clinical research will be directed at stopping amyloid deposition and increasing amyloid clearance.
- Protein misfolding is not only a characteristic of amyloidosis; it is also involved in many other disabling cardiac and neurologic degenerative diseases that interfere with healthy aging.

INTRODUCTION

The description of the autopsy of a young man in 1639 by Nicolaes Fonteyn, a Dutch physician and poet who lived in Amsterdam, was probably the first report of a patient with systemic amyloidosis. Since then, lardaceous changes in enlarged organs, such as the liver, spleen, heart, and kidneys, have drawn the attention of pathologists such as Rokitansky. In 1854, Rudolph Virchow was one of the first to use the term amyloid for this amorphous and hyaline change in tissue because of an iodine-staining reaction similar to that of starch (amylon; Greek for origin). Although it is now known that amyloid has nothing to do with starch, the term amyloid is still in use today. Bennhold

Funding Sources: None.
Conflict of Interest: Previous member of the Clinical Advisory Board of Neurochem; Chair of an External Safety Advisory Committee of GSK.
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Rheum Dis Clin N Am 39 (2013) 323–345
http://dx.doi.org/10.1016/j.rdc.2013.02.012 **rheumatic.theclinics.com**
0889-857X/13/$ – see front matter

introduced Congo Red staining in 1922 as a useful method to identify amyloid in tissue specimens. In 1927, Divry and Florkin described the characteristic green birefringence when Congo Red-stained amyloid was viewed under polarized light. In 1959, Cohen and Calkins detected the fibril nature of amyloid when viewed under the electron microscope.[1]

Amyloidosis is the name for 30 protein-folding diseases (**Table 1**), all characterized by extracellular deposition of a specific soluble precursor protein that aggregates in the form of insoluble fibrils. These rigid and unbranching fibrils, approximately 10 nm in diameter, are characterized by a molecular β-pleated sheet structure that is usually composed of peptides arranged in an antiparallel configuration. This structure of the fibrils is responsible for its insolubility, resistance to proteolysis, and binding affinity for Congo Red dye that shows a characteristic green birefringence when viewed under polarized light (**Fig. 1**).[2]

The aim of this article is to present a clinical overview of the different types of amyloidosis, disease manifestations, patient management, diagnosis, imaging techniques, treatment, and follow-up.

AMYLOID STRUCTURE AND PATHOGENESIS

Amyloidoses are protein-misfolding diseases in which small (parts of) proteins of about 10 to 15 kDa acquire an alternative and relatively misfolded state at minimum energy and subsequently aggregate into oligomers and polymers. Three mechanisms seem to operate independently or in combination: the precursor protein may have an intrinsic propensity to misfold that becomes evident with aging (wild-type transthyretin) or as high serum levels (serum amyloid A protein and immunoglobulin free light chains); a hereditary acquired mutated protein (transthyretin); and proteolytic remodeling of the precursor protein (β-amyloid precursor protein). Interaction with the extracellular matrix also seems to be important and may be related to preferential deposition of amyloid in some organs or tissues.[3]

Extracellular deposition of amyloid fibrils in organs and tissues results in tissue infiltration and swelling leading to progressive loss of function of the affected organ. Sometimes toxic effects, believed to be caused by toxic oligomers, have been observed (eg, in cerebral amyloid). These oligomers are nonfibrillar intermediate aggregates that are formed early in the process of fibril formation. Another constituent of all amyloid is serum amyloid P component (SAP), a glycoprotein that belongs to the pentraxin family and binds to all types of amyloid in a calcium-dependent way. SAP is highly protected against proteolysis and thus makes amyloid fibrils resistant to degradation. Apolipoprotein E is also a constituent of amyloid. Glycosaminoglycans (eg, heparan sulfate) are found in all types of amyloid and interact with extracellular matrix components such as laminin, entactin, and collagen IV. This interaction probably constitutes a scaffold that facilitates the initial phase of fibril nucleation and could have a targeting role in the localization of amyloid deposits in tissue.[3]

TYPES OF AMYLOIDOSIS

Deposition of amyloid is localized (ie, fibrils produced in and limited to 1 organ or site of the body) or systemic (ie, fibril deposition in various organs and tissues throughout the body). The current classification of amyloidosis (see **Table 1**) is based on the chemical characterization of the precursor protein.[2,4]

Organ-specific localized amyloidosis can be found in Alzheimer disease (β-protein in the plaques) and diabetes mellitus type 2 (amylin in the islands of Langerhans). The pathogenic role of amyloid deposition in these diseases is unclear.[4] Nodular

Table 1
Amyloid fibril proteins and their precursors in humans

Fibril Protein	Precursor Protein	Systemic or Localized	Acquired or Hereditary	Target Organs
AL	Immunoglobulin light chain	S, L	A	All organs except CNS
AH	Immunoglobulin heavy chain	S, L	A	All organs except CNS
Aβ2M	β2-microglobulin, wild type	S	A	Musculoskeletal system
	β2-microglobulin, variant	S	H	ANS
ATTR	Transthyretin, wild type	S, L	A	Heart mainly in men, tenosynovium
	Transthyretin, variants	S	H	PNS, ANS, heart, eye, leptomeninges
AA	(Apo) serum amyloid A	S	A	All organs except CNS
AApoAI	Apolipoprotein A I, variants	S	H	Heart, liver, kidney, PNS, testis, larynx (C-terminal variants), skin (C-terminal variants)
AApoAII	Apolipoprotein A II, variants	S	H	Kidney
AApoAIV	Apolipoprotein A IV, wild type	S	A	Kidney medulla and systemic
AGel	Gelsolin, variants	S	H	PNS, cornea
ALys	Lysozyme, variants	S	H	Kidney
ALect2	Leukocyte chemotactic factor-2	S	A	Kidney, primarily
AFib	Fibrinogen α, variants	S	H	Kidney, primarily
ACys	Cystatin C, variants	S	H	PNS, skin
ABri	ABriPP, variants	S	H	CNS
ADan[a]	ADanPP, variants	L	H	CNS
Aβ	Aβ protein precursor, wild type	L	A	CNS
	Aβ protein precursor, variant	L	H	CNS
APrP	Prion protein, wild type	L	A	CJD, fatal insomnia
	Prion protein, variants	L	H	CJD, GSS syndrome, fatal insomnia
ACal	(Pro)calcitonin	L	A	C-cell thyroid tumors
AIAPP	Islet amyloid polypeptide[b]	L	A	Islets of Langerhans, insulinomas
AANF	Atrial natriuretic factor	L	A	Cardiac atria
APro	Prolactin	L	A	Pituitary prolactinomas, aging pituitary
AIns	Insulin	L	A	Iatrogenic, local injection
ASPC	Lung surfactant protein	L	A	Lung
AGal7	Galectin 7	L	A	Skin
ACor	Corneodesmin	L	A	Cornified epithelia, hair follicles

(*continued on next page*)

Table 1
(*continued*)

Fibril Protein	Precursor Protein	Systemic or Localized	Acquired or Hereditary	Target Organs
AMed	Lactadherin	L	A	Senile aortic, media
AKer	Kerato-epithelin	L	A	Cornea, hereditary
ALac	Lactoferrin	L	A	Cornea
AOaap	Odontogenic ameloblast-associated protein	L	A	Odontogenic tumors
ASem1	Semenogelin 1	L	A	Vesicula seminalis

Abbreviations: ANS, autonomic nervous system; CJD, Creutzfeldt-Jakob disease; CNS, central nervous system; GSS, Gerstmann-Straussler-Scheinker syndrome; PNS, peripheral nervous system.
[a] ADan is the product of the same gene as Abri.
[b] Also called amylin.
Data from Sipe JD, Benson MD, Buxbaum JN, et al. Amyloid fibril protein nomenclature: 2012 recommendations from the Nomenclature Committee of the International Society of Amyloidosis. Amyloid 2012;19:167–70.

localized amyloid is an incidental finding and can be present in the skin (not only nodular but also macular amyloid and lichen amyloidosis), eyelid, conjunctiva, breast, larynx, bronchial tree, lung, and genitourinary tract. In most cases, low numbers of clonal plasma cells can be detected in the biopsy sample. Surgery is usually the treatment of choice. Local recurrence is frequent and can again be treated surgically.[5] Localized nodular skin amyloidosis is sometimes associated with Sjögren disease.[6] In contrast to localized amyloidosis, systemic amyloidosis leads to serious signs and symptoms caused by progressive disease in organs and tissues. There are many types of systemic amyloidosis (see **Table 1**), but 4 types are seen most frequently: AL, AA, ATTR, and $A\beta_2M$ amyloidosis.[4]

AL amyloidosis is the most common type. This disease is caused by a clonal plasma cell dyscrasia; it often occurs as low-grade clonal disease, sometimes multiple myeloma, and rarely non-Hodgkin lymphoma or Waldenström disease. The precursor of this type of amyloid is either lambda or kappa immunoglobulin free light chain. Clinical manifestations are diverse, such as cardiomyopathy, nephrotic syndrome, renal failure, hepatomegaly, splenomegaly, orthostatic hypotension, diarrhea, intestinal

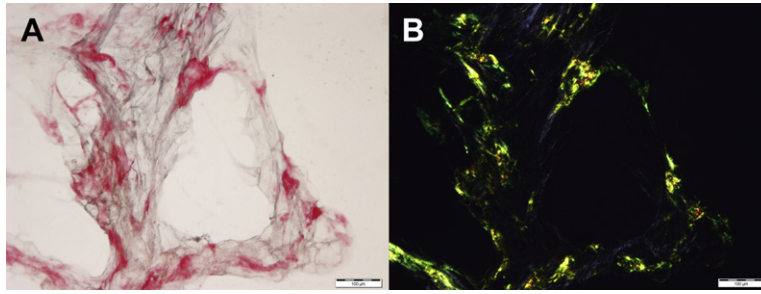


Fig. 1. A sample of abdominal subcutaneous fat aspirate containing amyloid deposits, stained with Congo Red. (*A*) Viewed in normal light, amyloid is stained red. Bar length is 100 μm. (*B*) Viewed in polarized light, amyloid shows apple-green birefringence (*collagen is bluish-gray*).

pseudoobstruction, peripheral neuropathy, autonomic neuropathy, arthropathy, carpal tunnel syndrome (CTS), bleeding, adrenal dysfunction, goiter, pulmonary problems, weight loss, fatigue, malaise, and glossomegaly.[7]

The second most common type is AA amyloidosis. This disease is caused by long-standing inflammation, such as rheumatoid arthritis, inflammatory bowel disease, chronic infections (eg, tuberculosis, osteomyelitis, leprosy), and hereditary autoinflammatory diseases (eg, familial Mediterranean fever, also called FMF). The precursor of this type is the HDL3-associated apolipoprotein serum amyloid A protein (SAA), an acute phase reactant. Signs of kidney disease, such as proteinuria (progressing to nephrotic syndrome) and loss of renal function (progressing to renal failure), are observed most frequently (in about 90% of cases), followed at a distance by autonomic neuropathy, bowel involvement, splenomegaly, hepatomegaly, goiter, and cardiomyopathy.[8,9]

The third most common type is ATTR amyloidosis. The familial form of this disease is caused by many autosomal dominantly inherited point mutations of the precursor protein transthyretin (TTR). Transthyretin is an acronym for the transport protein of thyroid hormone and retinol-binding protein. About 100 of these TTR mutations have been described, but most common is the TTR-Met30 mutation. Clinical manifestations are predominantly peripheral and autonomic neuropathy, but also cardiomyopathy, renal failure, and eye involvement (vitreous opacities) are frequently observed. Sometimes a severe cardiomyopathy is the initial presentation of the disease.[10] There is also a nonfamilial acquired form of ATTR amyloidosis. In this disease of old-aged men (rarely women), nonmutated (wild-type) TTR can also act as an amyloid precursor by a still unknown mechanism. This wild-type ATTR amyloidosis (formerly called senile systemic amyloidosis) is characterized by a slowly progressive cardiomyopathy, frequently associated with CTS, but without other neuropathy.[11]

The fourth type is Aβ_2M amyloidosis. This disease is caused by end-stage renal disease in which highly increased serum levels of β_2-microglobulin persist for years because β_2-microglobulin is not effectively cleared by dialysis. The disease is characterized by high serum levels of β_2-microglobulin and posttranslational modifications to be deposited as amyloid fibrils in predominantly osteoarticular tissues. CTS and shoulder pain are among the first manifestations, followed by large periarticular cysts and sometimes by pathologic fractures, or a destructive spondylarthropathy.[12,13] Recently, a hereditary Aβ_2M amyloidosis has been described, characterized by autonomic neuropathy and slowly progressive gastrointestinal symptoms.[14]

Other types of systemic amyloidosis are rare.[15] However, in patients without a family history of amyloid disease, a hereditary type of amyloidosis may be present.[16] In patients with almost exclusive kidney disease, other types should be considered, such as fibrinogen α,[17] lysozyme,[18] apolipoprotein A I, apolipoprotein A II, apolipoprotein A IV, and the recently described leukocyte chemotactic factor-2.[19] In patients with almost exclusive cardiac or neuropathic disease, the apolipoprotein A I type should not be overlooked. In patients with hepatic amyloid, the lysozyme and apolipoprotein A I type can be present. Apolipoprotein A I can be present in the skin and larynx. Gelsolin is typically found in lattice corneal dystrophy.[20]

EPIDEMIOLOGY

Even in developed countries, few incidence data have been collected systematically.[21] The data from 1 study about the incidence of AL amyloidosis in Olmsted County, Minnesota, are still used. In this study, the overall age-adjusted and sex-adjusted 95% confidence interval was 5.1 to 12.8 per million patients per year.[22] In

a recent Swedish study, the estimated incidence of AL amyloidosis was 3.2 per million per year and AA amyloidosis 2.0 per million per year.[23] The median age for AL and AA amyloidosis is between 55 and 60 years.[24] The prognosis of untreated patients is poor, as noticed in older studies; median survival was 6 to 12 months for AL amyloidosis and 3 to 4 years for AA amyloidosis.[24] The estimated median survival of untreated patients with hereditary ATTR amyloidosis is almost 10 years, although some patients may survive up to 15 years. An estimate of mortality is that 0.5 to 1.0 per 1000 persons die in the United Kingdom because of AL amyloidosis.[25] In most studies of patients with the AL type, the proportion of men is somewhat higher (about 1.1–1.3) than women. The reverse (more women than men) is observed for AA amyloidosis because of the high proportion of patients (mainly women) with underlying rheumatoid arthritis. In developing countries, the prevalence of AA amyloidosis is higher than AL amyloidosis because of the higher prevalence of associated underlying infectious diseases.

PATIENT MANAGEMENT

A stepwise approach is useful for the clinical management of a patient with systemic amyloidosis (**Box 1**).[26] If amyloidosis is suspected, the first step is to obtain histologic proof of amyloid, followed by a search for evidence of systemic amyloid deposition in the patient. The next 2 steps are to determine the type of amyloid with confidence followed by detection and (in AA and AL) quantification of the precursor protein in the blood. A thoughtful clinical evaluation should lead to useful knowledge on the severity of organ involvement, associated risks, prognosis, and an overview of all treatment options. The most effective treatment of the underlying precursor-producing process with acceptable risks and side effects should then be discussed with the patient. In the final step, a proactive plan is made to evaluate the chosen treatment during follow-up. In this plan, the effects of treatment must be monitored by assessing serum precursor levels and the amyloid load of the body. Although the amyloid load of the body cannot be measured directly, it is indirectly reflected by the function and size of affected organs and by specific imaging techniques.

DETECTION OF AMYLOID

Detection of amyloid should start with a reasonable clinical suspicion of amyloidosis. Clinical suspicion may increase on finding unexplained signs such as proteinuria, organomegaly (liver, spleen, or tongue), right-sided cardiac failure and/or biventricular hypertrophic cardiac walls, orthostatic hypotension, peripheral axonal polyneuropathy or autonomic neuropathy (especially the combination of the 2), and malabsorption.[4]

Box 1
Clinical management of a patient with systemic amyloidosis

- Definite proof of amyloid in tissue
- Convincing evidence for systemic amyloid deposition
- Unequivocal characterization of the type of amyloid
- Detection and/or quantification of the serum precursor protein
- Assessment of clinical severity of organ and tissue involvement
- Balanced choice of the most effective treatment with lowest risks
- Planned monitoring of the effect of treatment during follow-up

Sometimes, however, an alert pathologist performs a Congo Red stain after finding eosinophilic material in a tissue biopsy even without any clinical suspicion of amyloid. In that situation, the clinician has to deal with amyloid as a new and unsuspected finding.

Amyloid is a tissue-based diagnosis. Therefore, the diagnosis of amyloid is based on detecting its presence in tissue. The presence of amyloid is proved by a tissue specimen showing positive for Congo Red stain and the characteristic apple-green birefringence in polarized light (see **Fig. 1**). Subcutaneous abdominal fat tissue is easily accessible for this purpose and a video of such a fat aspiration procedure is available on the Web site at www.amyloid.nl.[27] Ample fat tissue can also be obtained by a simple surgical procedure.[28] Fat tissue stained with Congo Red has high sensitivity for AL, AA, and hereditary ATTR (up to 90%) and high specificity (almost 100%) if stained properly and viewed by experienced observers using a high-quality microscope with a good light source.[29] Sensitivity of rectum tissue for these types is about 80% and about 60% for bone marrow in AL. Although a biopsy of the affected organ (eg, kidney, liver, or heart) has the highest sensitivity (about 100%), it is recommended to start with a biopsy of a clinically uninvolved site, such as fat tissue, rectum, bone marrow, salivary gland, or gingiva, to avoid a biopsy of a vital organ and the associated risk of serious bleeding. In wild-type ATTR amyloidosis, the sensitivity of fat tissue analysis is a bit lower, about 73%.[30]

New staining methods for amyloid have been developed, such as luminescent conjugated oligothiophenes (LCOs). The first results showed that these molecules seem to bind to amyloid with higher sensitivity and greater selectivity than Congo Red, as determined by fluorescence microscopy and light polarization microscopy. Spectral profiles of tissue samples from 96 patients identified 3 nonoverlapping classes, which were found to match AA, AL, and ATTR types.[31] If these promising data can be confirmed by other investigators, this new staining technique might lead to improved detection of amyloid.

If amyloid has been found in a site specific for localized amyloidosis (genitourinary tract, eyelid, conjunctiva, larynx, and so forth.) it is recommended to screen for amyloid in another site of the body, such as fat tissue, rectum, bone marrow, or salivary glands, before diagnosing localized amyloidosis. Systemic amyloidosis is diagnosed if amyloid is present in 2 different sites of the body. There is consensus that systemic amyloidosis is also present if amyloid has been detected in only 1 site of the body in combination with a classic picture of amyloidosis (**Table 2**) at an alternate site.[32]

TYPING OF AMYLOID WITH CONFIDENCE

After detection of amyloid, the specific type of amyloid should be characterized with confidence. In most cases the type of amyloid can be assumed because of the medical history and clinical picture. Nevertheless, even in patients with strong clinical evidence for a particular type of amyloid, it is still necessary to search for solid evidence of the specific type of amyloid involved because incorrect typing of amyloid can have severe clinical consequences. The prognosis and treatment modalities differ enormously among the 4 main types of systemic amyloidosis.

The usual method of typing amyloid is by immunohistochemistry of a biopsy sample using specific antibodies. In AA amyloidosis, this technique is sufficient provided sensitive and specific monoclonal antibodies are used. However, immunohistochemistry is less reliable in ATTR amyloidosis and is frequently even useless to demonstrate AL amyloidosis.[33,34] The absence of a positive family history does not exclude ATTR

Table 2
Organ involvement: positive biopsy at an alternate site[a] and a positive organ criterion

Organ	Criterion
Kidney	24-h urine protein >0.5 g/d, predominantly albumin
Heart	Echo: mean wall thickness >12 mm, no other cardiac cause
Liver	Total liver span >15 cm in the absence of heart failure or alkaline phosphatase >1.5 times institutional upper limit of normal
Nerve	Peripheral: clinical; symmetric lower extremity sensorimotor peripheral neuropathy Autonomic: gastric-emptying disorder, pseudoobstruction, voiding dysfunction not related to direct organ infiltration
Gastrointestinal tract	Direct biopsy verification with symptoms
Lung	Direct biopsy verification with symptoms Interstitial radiographic pattern
Soft tissue	Tongue enlargement, clinical Arthropathy Claudication, presumed vascular amyloid Skin Myopathy by biopsy or pseudohypertrophy Lymph node (may be localized) Carpal tunnel syndrome

[a] Alternate sites available to confirm the histologic diagnosis of amyloidosis: fine-needle abdominal fat aspirate and/or biopsy of the minor salivary glands, rectum, or gingiva.

Data from Gertz MA, Comenzo R, Falk RH, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18–22 April 2004. Am J Med 2005;79:319–28.

amyloidosis, as demonstrated by several sporadic cases.[16] Therefore, a TTR mutation has to be confirmed by DNA analysis in ATTR amyloidosis. The exception to this rule is wild-type ATTR amyloidosis in which, by definition, a TTR mutation is absent.

In patients with AL amyloidosis, an underlying monoclonal plasma cell dyscrasia with overproduction of either lambda or kappa light chains can usually be detected by investigating bone marrow (clonal dominance by immunophenotyping of plasma cells), urine (Bence Jones proteins, immunofixation of concentrated urine), and blood (M-protein, immunofixation, and, most important of all, by the free light chain assay). Detection of a monoclonal gammopathy of undetermined significance does not exclude other types than AL amyloidosis. The clinical pictures of ATTR amyloidosis and AL amyloidosis are sometimes quite similar, for example, in cases with polyneuropathy, autonomic neuropathy, cardiomyopathy, or CTS. If such a clinical picture is present, it is not sufficient to detect the presence of a plasma cell dyscrasia; it is also necessary to exclude a TTR mutation before reliably concluding AL amyloidosis.[16] In elderly men, the choice between wild-type ATTR and AL amyloidosis may be hard to make if a slightly increased serum free light chain is detected in a patient with cardiomyopathy as the sole disease manifestation.

New proteomics techniques have been developed to chemically analyze the protein composition of tissues. The application of proteomics seems to be a promising tool for reliable typing of amyloid.[35–39] These techniques, with high sensitivity and high specificity, are especially helpful to distinguish between AL and ATTR amyloid. One of these elegant techniques combines specific sampling by laser microdissection with the analytical power of proteomic analysis based on tandem mass spectrometry.[37]

However, currently these sophisticated, expensive, and time-consuming techniques are only available in highly specialized centers, so for the time being immunohistochemistry remains the standard procedure for typing of amyloid.[40]

DISEASE MANIFESTATIONS

Although amyloidosis must have been present for a long time (often more than a year, looking back to the start of the first symptoms), the disease goes unnoticed until alarming symptoms appear relatively late in its course. Nonspecific complaints such as fatigue and weight loss gradually appear and can be debilitating, but are often noticed only after disease progression leads to more specific signs, such as edema, dyspnea, bleeding, or orthostatic hypotension. A limited overview of the diverse disease manifestations of the main types of amyloidosis[4,7–10,24] is presented in this section.

Renal disease is often seen in both AL and AA amyloidosis (in about 70%–90% of cases) and rarely in ATTR amyloidosis. Sometimes the presentation is unremarkable as asymptomatic proteinuria, but often it appears dramatically as frank nephrotic syndrome or severe renal failure. The insidious nature of amyloidosis keeps the disease unnoticed for a while until edema appears as a first sign of the disease.

Edema, however, is also a presenting sign of cardiac disease. Signs and symptoms of right-sided heart failure (edema, increased jugular venous pressure, third heart sound, and hepatomegaly) are often seen. The clinical picture is that of a constrictive cardiomyopathy, initially affecting the inflow of the heart more than the outflow. The ejection fraction and cardiac size on the chest radiograph usually remain in the normal range for a long time. Hypotension is often a prominent feature. Rapidly progressive cardiac involvement is frequently part of the clinical picture of AL amyloidosis (in about 40%–60% of cases), whereas slowly progressive cardiomyopathy is usually seen in ATTR amyloidosis. Cardiomyopathy is infrequent (about 5% of cases) in AA amyloidosis. A characteristic low-voltage pattern or a pseudoanteroseptal infarction pattern is sometimes visible on the electrocardiogram. The combination of a low-voltage pattern and thickened ventricular walls (left and right) on cardiac ultrasonography is pathognomonic for an infiltrative cardiomyopathy such as amyloidosis. Accumulation of amyloid in the coronary arteries may lead to (often atypical) angina or infarction. Ventricular tachycardia is a frequent and dangerous complication of AL amyloidosis. Conduction disturbances are often seen in ATTR amyloidosis and can necessitate insertion of a pacemaker in the long term. Increased serum N-terminal pro-brain natriuretic peptide (NT-proBNP) and troponin-T concentrations can reveal asymptomatic cardiac involvement and help to assess associated risks before the start of any treatment.[41] Midregional proadrenomedullin (MR-proADM) seems to be a new and powerful prognostic marker in AL amyloidosis, which may not only reflect cardiac dysfunction but also widespread systemic disease, and can be combined with troponin-T to detect patients at risk of early death.[42]

Hepatomegaly is rare in AA amyloidosis and is not seen in ATTR amyloidosis. It is sometimes a presenting feature of AL amyloidosis, and the characteristic biochemical profile of intrahepatic cholestasis shows an increase in γ-glutamine transpeptidase, followed by alkaline phosphatase and the bilirubin concentration. It is not always easy to distinguish amyloid hepatomegaly from liver enlargement secondary to right-sided cardiac failure. Splenomegaly is seen in about 5% of patients with AL amyloidosis and hyposplenism (identified by Howell-Jolly bodies or target cells) is present in about 25%. Malabsorption, pseudoobstruction, ulceration, and gastrointestinal

bleeding are infrequent but severe manifestations of bowel involvement. Gastroparesis, constipation, and diarrhea are more common and seem to be caused by autonomic neuropathy. Sometimes the clinical picture is worsened by bacterial overgrowth.

Peripheral sensory polyneuropathy, with ascending symptoms of numbness, paresthesia, and pain, is frequently observed in AL and ATTR amyloidosis but is extremely rare in AA amyloidosis. Autonomic neuropathy is seen in all types of amyloidosis but is most frequent and severe in AL and ATTR amyloidosis; it can lead to orthostatic hypotension, impotence, bladder voiding disturbances, early sensation of fullness, nausea, vomiting, diarrhea, and constipation.

Although CTS is a neuropathy of the median nerve and can therefore be part of a generalized polyneuropathy, it is usually caused by entrapment caused by synovial thickening through amyloid. CTS can be a manifestation of amyloid arthropathy (with the characteristic shoulder-pad sign and pseudoarthritis of the small hand joints and wrists) in AL and $A\beta_2M$ amyloidosis. CTS is sometimes seen in wild-type ATTR amyloidosis. A diversity of other manifestations may be present in AL amyloidosis, such as waxy skin and skin nodules, easy bruising (vascular wall fragility), periorbital purpura (raccoon eyes), coagulation abnormalities caused by Factor X deficiency (as a result of increased removal by binding to amyloid), macroglossia (in about 20% of cases) with indentations and submandibular swelling, dystrophic nails, taste disturbance, hoarseness, jaw claudication, musculopathy (pseudohypertrophy or muscular dystrophy), bladder bleeding, lymphadenopathy, subclinical hypothyroidism, and hypoadrenalism. Pulmonary amyloidosis is characterized by a reticulonodular pattern on the chest radiograph and is rare. Pleural amyloidosis goes often undetected, but becomes visible as rapidly progressive, relatively large, and diuretics-resistant pleural effusions, usually during concomitant cardiac failure. Vitreous opacities may be present, but only in ATTR amyloidosis in some of the TTR mutations. Central nervous system involvement is unusual. Meningeal amyloidosis is only seen in some mutations in ATTR amyloidosis. Involvement of the pituitary gland has been described, but is rare.[43–45] Ischemic stroke may be seen and is often caused by an embolism derived from the affected heart.[46]

IMAGING OF AMYLOIDOSIS

Amyloidoses are difficult diseases to diagnose. Their insidious appearance, the diverse ways they present to many medical specialists, the often severely affected organ function at diagnosis and the dangerous combinations of vital organs affected all demand that the treating physician obtains a clear overview of the amyloid disease. The clinician needs relevant information about the function and size of affected vital organs. This information is essential both for diagnosis and for disease monitoring during follow-up. Blood tests can be used to assess organ function, whereas imaging can be used to assess size and function of organs. Ultrasonography of the abdomen, heart, and musculoskeletal system, magnetic resonance imaging (MRI) of the heart, bone scintigraphy, and SAP scintigraphy are all useful imaging techniques in selected cases.

Ultrasonography, computed tomography (CT), and MRI are well-known techniques to evaluate the size of the kidneys, liver, and spleen. Musculoskeletal ultrasonography is useful in $A\beta_2M$ amyloidosis.[47] Cardiac ultrasonography is the method of choice to quickly investigate the presence of myocardial sparkling, restriction to diastolic filling, ejection fraction, and the thickness of the interventricular septum, left ventricular posterior wall, and right ventricular wall. MRI of the heart can show global gadolinium late enhancement in a subendocardial distribution that is highly sensitive and specific

for the identification of cardiac involvement.[48] [^{123}I]metaiodobenzylguanidine may help to detect cardiac sympathetic denervation.[49] Aprotinin scintigraphy has been used successfully for the identification of cardiac involvement in AL amyloidosis.[50] A disadvantage of this tracer, however, is the potential infectious risk, because it is a polypeptide derived from bovine lung tissue. Technetium Tc 99m pyrophosphate and diphosphonate sometimes bind to amyloid and have been used as imaging agents in amyloidosis, especially for detecting cardiac involvement in ATTR amyloidosis (**Fig. 2**).[51,52] A similar tracer (3,3-diphosphono-1,2-propanodicarboxylic acid) has also been shown to be useful for this purpose.[53,54]

SAP scintigraphy was developed in London by Hawkins and colleagues[55] to detect and identify the distribution of amyloid in systemic amyloidosis. SAP binds in a calcium-dependent way to all amyloid deposits. Scintigraphy with ^{123}I-labeled SAP shows

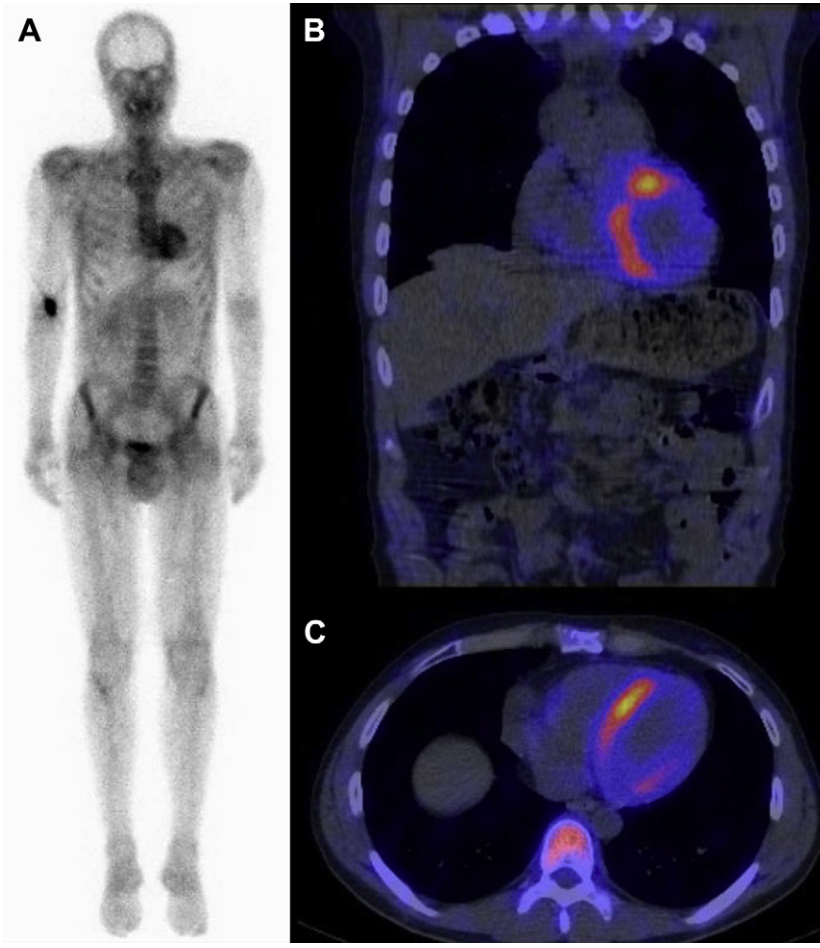


Fig. 2. Technetium Tc 99m-methylene diphosphonate bone scintigraphy in a 31-year-old man with ATTR amyloidosis. (*A*) Total body scan with increased cardiac uptake and soft tissue uptake. The skeletal uptake is relatively diminished. (*B*) Coronal and (*C*) transverse single photon emission-computed tomography/CT images of the heart showing increased uptake in the ventricular walls.

specific uptake in the liver, spleen, kidneys, adrenals, bone marrow, and joints (**Fig. 3**).[55–57] The heart cannot be visualized with this technique. Sensitivity of the SAP scan for AL and AA amyloidosis is about 90%, but it is only 48% for hereditary ATTR amyloidosis. Specificity is about 90%. SAP is isolated and purified from serum of healthy donors and the potential infectious risk is probably the major reason why this useful technique is currently being used only in the United Kingdom and The Netherlands. Measurement of ^{123}I SAP retention in the body after 24 or 48 hours provides a rough quantitative estimate of the amyloid load in the patient.[58,59] Recently, an amyloid fibril–specific monoclonal antibody, 11-1F4, has shown promising results in detecting AL amyloidosis when used as a tracer in positron emission tomography/CT.[60]

TREATMENT

The current basis for treatment is the so-called precursor-product concept. The central idea of this concept is that further growth of amyloid deposits will stop when the supply of necessary precursors is stopped. Thus, it is important to diagnose amyloidosis early and start treatment as early as possible to stabilize the disease and prevent ongoing progression.

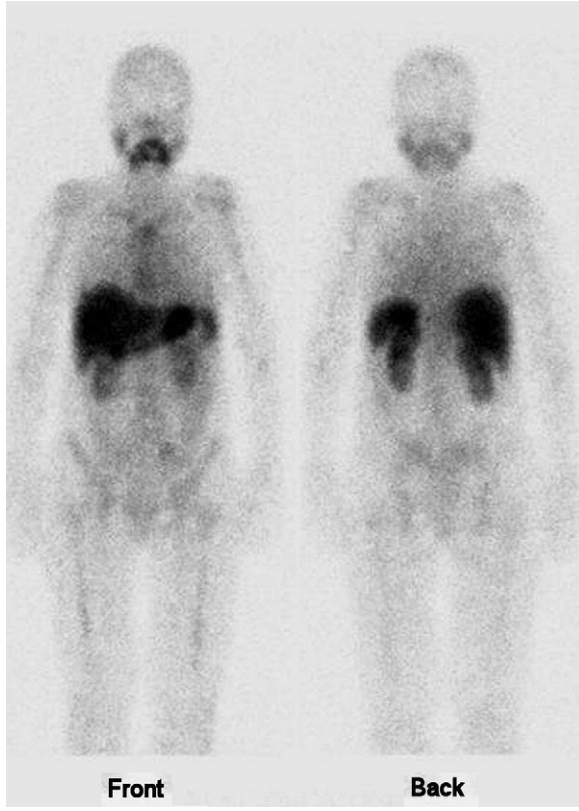


Fig. 3. Total body SAP scintigraphy (anterior and posterior images) of a 60-year-old woman with systemic amyloidosis (probably AL type) with increased uptake (++) in the liver, spleen, and kidneys and (+) bone marrow. Minor nonspecific uptake can be seen in the parotid glands, nasopharynx and stomach.

Treatment for AA amyloidosis is aimed at decreasing SAA serum levels to normal basal values (<3 mg/L).[8] If this level can be reached and maintained at less than 10 mg/L, the 10-year survival rate increases to 90%; when the SAA levels are more than 10 mg/L, this figure is less than 40%.[61] The only way to achieve a normal basal serum value of SAA is by complete suppression or eradication of the underlying chronic inflammatory disease. This can be realized in patients with infectious diseases such as tuberculosis, leprosy, recurrent pulmonary infections, and osteomyelitis through eradication of the infection by antibiotic treatment sometimes combined with surgery. The treatment of chronic inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, and Crohn disease has improved dramatically in the last decades. Because of the introduction of more effective antiinflammatory drugs such as methotrexate and biologics, especially those directed against tumor necrosis factor (TNF) and interleukin-1 (IL-1), effective suppression of SAA to low or even normal serum concentrations has become a realistic goal. In addition, the autoinflammatory diseases such as FMF, TNF-receptor associated periodic syndromes, the hyper-IgD syndrome, and cryopyrin-associated periodic syndromes often respond well to some of these biologics, especially anakinra, which is directed against IL-1.[62] A promising new biologic is tocilizumab, an anti-IL6 receptor antibody that directly suppresses the production of C-reactive protein and SAA by the liver.[63]

Treatment for AL amyloidosis is aimed at eradicating the underlying plasma cell dyscrasia by chemotherapy, and return the abnormally increased level of kappa or lambda free light chain in the blood to the normal range.[64] High-dose melphalan (HDM) followed by autologous stem cell transplantation (ASCT) in eligible patients has shown considerable benefits.[65] Median survival in this low-risk group, the ones illegible for ASCT, was 4.6 years. However, 1 randomized clinical trial questioned the favorable results of HDM followed by ASCT.[66] Meanwhile, many more studies of novel drugs, such as thalidomide, bortezomib, lenalidomide, pomalidomide, and MLN9708, have shown clear effects, often with the best effects in combination with dexamethasone.[67] In a review by Gatt and Palladini,[67] a state-of-the-art treatment schedule is presented for low-risk, intermediate-risk and high-risk patients. The survival of responding patients has increased and recent reports of patient cohorts on long-term survival are encouraging. However, early deaths due to advanced, irreversible cardiac dysfunction at presentation remain a huge unsolved problem. Median survival of such untreated patients with advanced cardiac involvement is 3 to 6 months and does not really change with treatment. The debate concerning the most effective and least dangerous treatment regimens will probably continue for the next few years. The concept of striving for normalization of the free light chain involved is still unchallenged and this normalization seems to result in actual regression of the amount of amyloid in tissue, as has been shown in fat tissue.[68]

Until recently, the only treatment for patients with hereditary ATTR amyloidosis was liver transplantation with the aim of removing the source of 99% of the mutated TTR in the circulation.[69] However, this approach is not always successful because ATTR amyloid sometimes progresses in the heart after liver transplantation (**Fig. 4**). For this reason, patients with late disease onset (often men with cardiomyopathy) and non–TTR-Met30 mutations are less suitable for liver transplantation.[69,70] The amyloid fibril composition also seems to predict progressive cardiomyopathy after liver transplantation.[71] The 10-year survival of the TTR-Met30 patients after liver transplantation is currently about 85%.[69]

In Aβ_2M amyloidosis, high-flux membranes and adsorption columns have been studied in hemodialysis in an attempt to lower the β_2M serum concentrations. Kidney

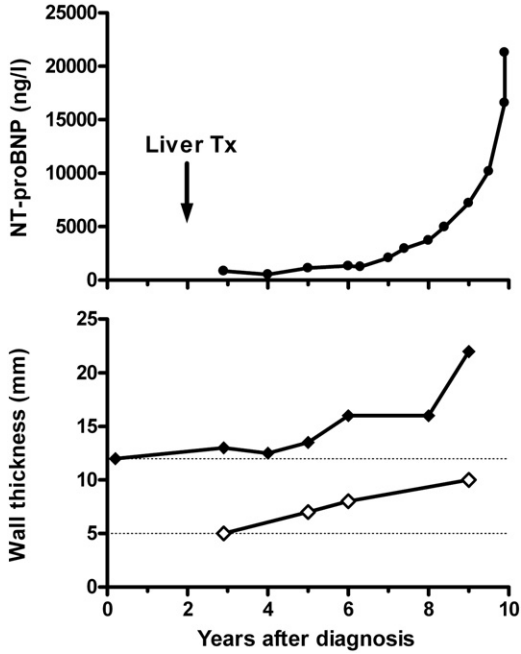


Fig. 4. A 51-year-old woman with hereditary ATTR amyloidosis who received a liver transplant (Liver Tx) 2 years after diagnosis. Progressive cardiomyopathy reflected by an increase of NT-proBNP (*black circles*), mean left ventricular wall size (*black diamonds*), and right ventricular wall size (*white diamonds*). The dotted lines represent the upper limits of the right ventricular wall diameter (5 mm) and left ventricular wall diameter (12 mm).

transplantation, however, remains the treatment of choice. After transplantation, the amyloidosis seems to stabilize; the β_2M serum levels decrease to normal, bone pain and stiffness decrease, the cystic lesions do not increase further in size, but regression of amyloid deposits has not been reported.[72]

Apart from treating the underlying precursor-producing process, it is also important to provide supportive treatment for decreased organ function caused by amyloid deposition. Involvement of more than 1 vital organ frequently results in a mixture of serious problems. It is often difficult and sometimes impossible to find an acceptable solution for all problems. To get the best results of supportive treatment for the individual patient with amyloidosis, it is necessary that all medical specialists involved collaborate closely and that 1 specialist coordinates the collective efforts.

DISEASE MONITORING

Maintaining a good overview of the effect of treatment is important for such an intangible disease as systemic amyloidosis. The accumulation of amyloid is expected to stop after successful elimination of the precursor supply and the tissue itself tries to remove amyloid. Repeated measurements are useful to monitor the effect (or lack of effect) of treatment. Two different processes need to be monitored (**Box 2**).

The first process is that of the underlying production of the precursor proteins SAA, free kappa or lambda light chain, and (mutated) TTR in AA, AL, and ATTR amyloidosis, respectively. After successful treatment, the SAA levels should decrease and remain

Box 2
Monitoring of systemic amyloidosis during follow-up using a set of core data. Not all items are always indicated; the choice depends on type of amyloid and patient characteristics

Frequent (3–6 times per year):	
Precursor	SAA and CRP (AA)
	Kappa or lambda free light chain, M-protein, Bence Jones in urine (AL)
Serum	Urea, creatinine, albumin
	Total bilirubin, alkaline phosphatase, gamma glutamyl transpeptidase
	N-terminal pro-brain natriuretic peptide, troponin-T
Urine	Endogenous creatinine clearance
	Protein/24 h, protein/creatinine ratio
Infrequent (once per year, two years, or if indicated):	
Precursor	Bone marrow biopsy (AL) to verify complete response or suspicion of relapse
Imaging	Echocardiography (ventricular wall thicknesses, ejection fraction)
	Abdominal echography (sizes of liver, spleen, and kidneys)
	Diphosphonate scintigraphy (ATTR)
	SAP scintigraphy (AA and AL)
Function	ECG, Holter monitoring for 24 h
Tissue	Amyloid grade (or amyloid protein concentration) in subcutaneous fat tissue

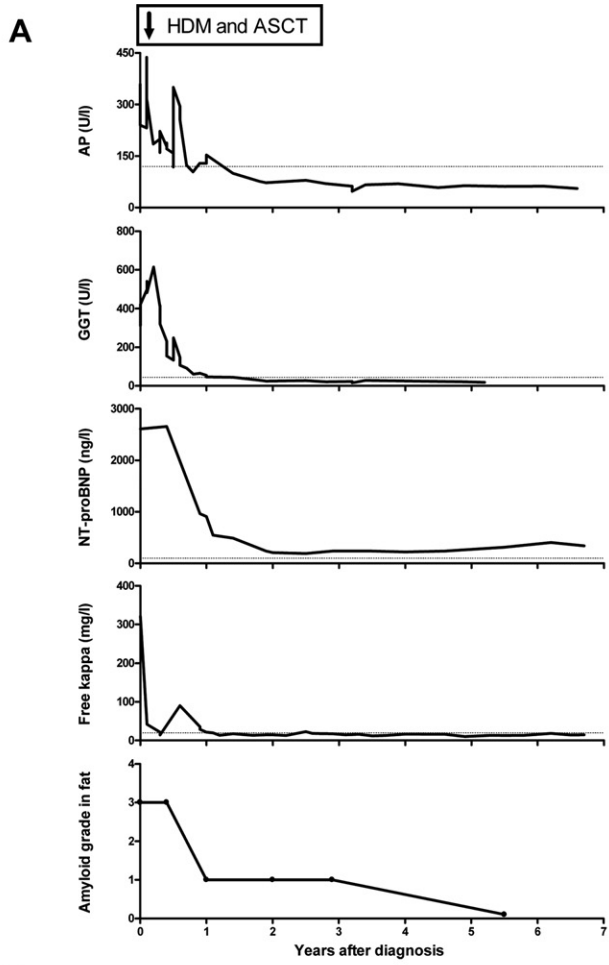
at less than 3 mg/L continuously, the free kappa and lambda levels and the kappa/lambda ratio should definitely decrease within the reference ranges, and mutant TTR should no longer be detectable in the blood.

The second process is that of amyloid accumulation, the so-called clinical amyloid load. Quantitative clinical abnormalities are available for monitoring this clinical amyloid load, such as serum albumin, alkaline phosphatase, bilirubin, NT-proBNP, troponin, creatinine clearance, proteinuria, ventricular wall thickness, ejection fraction, conduction and rhythm, heart rate variability, Ewing battery results of autonomic function, and the sizes of enlarged organs, such as the liver, spleen, and kidneys. Imaging techniques and subcutaneous fat tissue samples can be used for monitoring (**Fig. 5**). At a consensus meeting in Tours in 2004, a set of response criteria for systemic AL amyloidosis was accepted by the international amyloidosis community,[32] with the addition of some modifications after the last consensus meeting in Groningen in 2012.[67]

CURRENT PERSPECTIVES

The precursor-product concept helps in understanding why the current treatment aimed at normalization of the precursor protein is important to prevent further accumulation of amyloid. New developments in this area include antisense[73] and RNAi[74] treatment. Both treatments suppress TTR production by the liver resulting in low TTR serum concentrations. Most of the current research, however, has changed its focus to the development of new drugs to stabilize precursor proteins, interfere with amyloid deposition, or stimulate amyloid removal.

New drugs are under investigation for ATTR amyloidosis, such as diflunisal and tafamidis, that stabilize the conformation of TTR in the circulation and interfere with deposition of amyloid. Both diflunisal and tafamidis stabilize the TTR tetramer in blood in vitro and inhibit the tetramer in breaking up into amyloidogenic dimers and monomers.[75] A clinical trial of tafamidis has recently been published and shows an inhibitory effect on the progression of polyneuropathy in patients with ATTR amyloidosis.[76] A clinical trial of diflunisal comprising 130 patients was completed at the end of 2012, so the results are expected soon.[77] A study of doxycyclin in combination with


A
HDM and ASCT
AP (U/l)
GGT (U/l)
NT-proBNP (ng/l)
Free kappa (mg/l)
Amyloid grade in fat
Years after diagnosis
B
Start
1 year
3 years
6 years

tauroursodeoxycholic acid, a biliary acid, in mice showed that this combination was capable of stimulating removal of ATTR amyloid deposits.[78] A phase II study of this drug combination has been started in patients with ATTR amyloidosis.[79]

Eprodisate, another promising drug, has shown an inhibitory effect on the progression of kidney disease in patients with AA amyloidosis in a first clinical trial.[80] This drug interferes with the binding of SAA to glycosaminoglycans in tissue.[81] Although the magnitude of the clinical effects of tafamidis in ATTR amyloidosis and eprodisate in AA amyloidosis is moderate, the real relevance of both studies is that they show proof of concept that interference with the formation and deposition of amyloid is a realistic goal. Epigallocatechin-3-gallate (EGCG), a green tea extract, seems to have an inhibitory effect on the formation of AL and ATTR amyloid.[82–84] A clinical trial has started to evaluate a possible role of EGCG in promoting regression of residual cardiac damage in patients with AL amyloidosis who have successfully completed chemotherapy.[67]

An interesting development is the combined use of a drug called CPHPC and anti-SAP antibodies. CPHPC (the abbreviation of (*R*)-1-[6-[(*R*)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid) is a drug that effectively depletes SAP from the circulation.[85] This mechanism possibly stops accumulation of amyloid and may be useful for all types of systemic amyloidosis, although fibrinogen-derived amyloidosis seems to respond best.[86] Vaccination against amyloid has been attempted in the past with poor results. Early vaccination research was primarily focused on the induction of antibodies against conformational epitopes that were believed to be present in all types of amyloid.[87] The Royal Free group in London recently demonstrated that CPHPC followed by anti-SAP antibodies resulted in a quick removal of almost all AA amyloid from the tissues in mice with AA amyloidosis.[88] If this dramatic effect on amyloid can be replicated in humans, it may lead to a major change in the prognosis for patients with all types of systemic amyloidosis.

HEALTHY AGING AND PROTEIN MISFOLDING

Autopsy data suggest that supercentenarians (110 years or older) die in about 70% of cases from ATTR amyloidosis-related causes.[89] Neurodegenerative diseases such as Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, and Huntington disease, in all of which protein misfolding is a prominent feature, emerge with aging. The induction of aggregation involves a crystallizationlike seeding mechanism by which a specific protein is structurally corrupted by its misfolded conformer.[90] Seeding is also described in other forms of amyloidosis and, although the clinical consequences do not seem to be prominent, this prionlike behavior is an aspect of these

Fig. 5. A 62-year-old woman with systemic AL kappa amyloidosis associated with multiple myeloma. She was treated with HDM followed by ASCT. (*A*) Follow-up of serum concentrations of alkaline phosphatase (AP), γ-glutamyl transpeptidase (GGT), NT-proBNP, and the kappa free light chain. The bottom part shows the semiquantitative grading of amyloid in fat tissue (ranging from 0 to 4+). Dotted lines display the upper limit of the reference control values. (*B*) Follow-up of SAP scintigraphy (*anterior view*) at the start of treatment and 1, 3, and 6 years thereafter. At the start, intensive uptake in an enlarged liver (+++), spleen (+++) and in bone marrow (++) is visible. After 1 year, diminished bone marrow and splenic uptake (+) is visible and the liver uptake has become normal except for a small upper segment of the right lobe. After 3 and 6 years, some splenic uptake persists (+), the localized liver uptake diminishes slowly, and the bone marrow uptake has disappeared. Some nonspecific uptake or excretion can be seen in thyroid, stomach, and bladder (and minimal extravasation of blood or tracer at the injection site near the left elbow after 1 and 3 years).

protein-misfolding diseases that certainly deserves serious attention.[91,92] In common cardiac diseases, such as pathologic cardiac hypertrophy and dilated and ischemic cardiomyopathies, misfolded proteins have a direct causative role.[93] Protein misfolding is not only a characteristic of some obscure amyloid diseases but is a much broader phenomenon that is involved in many other degenerative diseases closely associated with aging.[94] Therefore, a better understanding of the nature and pathogenesis of all types of amyloidosis may also increase our knowledge of these common and disabling degenerative diseases that interfere so much with healthy aging.

ACHIEVEMENTS

The amyloidoses are fascinating representatives of a new disease category of protein-misfolding diseases. Hundred years ago it was a mystery why so different diseases as multiple myeloma and tuberculosis could end up with organs massively filled with apparently similar amyloid. Often, however, amyloidosis appeared in a patient without any clear cause, and was therefore thought to be primary. Occasionally the disease was found in succeeding generations of a family. But diagnostic possibilities improved steadily. The iodine sulphuric staining test was replaced by metachromatic stains, such as Congo red, in order to better identify amyloid in tissue. It was the introduction of biopsies that enabled clinicians to diagnose amyloid during life, although biopsies were used restrictively until the mid twentieth century. But the major breakthrough came with the work of Pras in 1968, who succeeded in isolating amyloid fibrils by extraction with distilled water.[95] Since that time the amyloid protein became accessible for chemical analysis. The amyloid proteins were chemically characterized, amyloid precursor proteins were detected in blood, and many DNA mutations were connected to familial types of amyloidosis.

The current classification of amyloidosis is in a way a monument of the recent analytical and clinical research. Nowadays a patient suffering from systemic amyloidosis can be diagnosed and typed rapidly with minimal discomfort. The patient undergoes a clinical assessment consisting of blood analyses, function tests, and imaging techniques that yields a quick overview of the severity of organ disease and associated risks. Treatment possibilities range widely from several biologic modifiers, novel cytostatic drugs, or drugs specifically designed for amyloidosis to transplantation of liver or bone marrow. Monitoring the effects of treatment during follow-up has improved and enables the clinician to assess early whether or not the amyloidosis responds to the chosen treatment.

CURRENT CHALLENGES

Despite these favorable developments, however, the prognosis remains grim for many patients suffering from systemic amyloidosis. The reasons are manifold, but the current main challenges are lack of awareness and loss of precious time in waiting for - often ineffective - precursor elimination.

Lack of awareness leads to late detection and far advanced disease at presentation, often severe cardiac disease or multi-organ disease. Doctors should think of amyloidosis - or their computers should suggest it - if key symptoms for the diagnosis are present. This is especially true if more symptoms than one are present. Typical key symptoms are right sided cardiac failure, proteinuria or renal failure, organ swelling (of tongue, myocardium, liver, or spleen), polyneuropathy, or autonomic neuropathy that are all unaccounted for. In many such cases looking for an elevated serum free light chain and performing an abdominal fat aspiration may speed up the diagnosis by months.

Waiting until the precursor has decreased to normal levels is sometimes impossible, because the disease progresses meanwhile. Many patients with severe cardiac AL amyloidosis have not had any profit of improved treatment, because they die from disease progression in the first months after diagnosis. And even sometimes the amyloidosis progresses despite successful reduction or even elimination of the precursor. This is frequently observed in some types of ATTR amyloidosis after liver transplantation, because wild-type TTR becomes deposited in the heart instead of mutant TTR. There is a need for reliable methods to measure the actual production and deposition of amyloid. And treatment tools need to be developed that stop amyloid deposition immediately after detection as well as additional tools that effectively help to remove amyloid from the body. So, although much has been achieved in the last fifty years, there is much left for fundamental and clinical researchers to improve the prospects of patients suffering from these deadly, but also fascinating amyloid diseases.

ACKNOWLEDGMENTS

I would like to thank Johan Bijzet BSc and Andor Glaudemans MD for their kind support in obtaining material for the figures.

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